

# Surveillance of Transmitted HIV-1 Drug Resistance in Gauteng and KwaZulu-Natal Provinces, South Africa, 2005–2009

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**Surveillance of human immunodeficiency virus type 1 transmitted drug resistance (TDR) was conducted among pregnant women in South Africa over a 5-year period after the initiation of a large national antiretroviral treatment program. Analysis of TDR data from 9 surveys conducted between 2005 and 2009 in 2 provinces of South Africa suggests that while TDR remains low (<5%) in Gauteng Province, it may be increasing in KwaZulu-Natal, with the most recent survey showing moderate (5%–15%) levels of resistance to the nonnucleoside reverse transcriptase inhibitor drug class.**

South Africa has adopted a public health approach to antiretroviral (ART) delivery using standardized treatment options and management protocols. By the end of 2009, an estimated 970 000 South Africans infected with human immunodeficiency virus (HIV) type 1 were accessing ART through the public health-care system [1]. The first-line regimen at this time was stavudine, lamivudine, and efavirenz or nevirapine, with ritonavir-boosted lopinavir being used for treatment of infants and as a second-line regimen in adults.

The annual antenatal survey (ANSUR), conducted by the National Department of Health, is an anonymous, unlinked cross-sectional survey that estimates HIV prevalence using blood specimens taken from pregnant women aged 15–49 years attending 1 of the 1457 public health sector antenatal clinics across all 9 provinces in South Africa. Between 2005 and 2009, the number of pregnant women participating in these surveys

increased from 16 510 to 32 861 [2, 3]. The majority of women were from the Gauteng (GP) (21.9%) and KwaZulu-Natal (KZN) (20.5%) provinces, with roughly half being between ages 15 and 24 years. In 2009, the national HIV prevalence estimate was 29.4% with 29.8% in GP and 39.5% in KZN.

Emergence of HIV drug-resistant strains is an inevitable consequence of ART, which has been potentially exacerbated by rapid up-scaling of population-based treatment regimens. The World Health Organization (WHO) recommends surveillance for transmitted drug resistance (TDR) in countries where ART has been available for >3 years among individuals likely to be recently infected, such as women aged <25 years in their first pregnancy [4]. This minimum-resource method analyzes ≤47 specimens from individuals consecutively identified as HIV-infected to categorize TDR as low (<5%), moderate (5%–15%), or high (>15%) [5]. Levels of TDR have remained low despite extensive HIV drug resistance (HIVDR) being documented among patients failing first-line therapies in resource-limited countries, [6].

An earlier survey conducted in GP between 2002 and 2004 [7] showed low levels of TDR, which was not unexpected given that the national ART program began in April 2004. In this study, we performed

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a retrospective analysis of HIVDR using ANSUR specimens from GP and KZN, obtained between 2005 and 2009, and spanning the time during which the national ART program expanded significantly.

## METHODS

### ANSUR Specimens

All participants were from GP or KZN and were part of the 2005–2009 ANSUR. Anonymized demographic data were recorded on a standardized collection form. All individuals in this analysis met the inclusion criteria as defined by the WHO guidelines for classification of TDR (primigravida female, aged <25 years) [4]. Data from 9 surveys were available for analysis. (The KZN-2006 survey was not included.) Ethical approval for HIVDR testing was obtained from the University of the Witwatersrand Human Research Ethics Committee.

### Specimen Collection and HIV Testing

Serum specimens were collected during routine antenatal care and tested for HIV infection by enzyme-linked immunosorbent assay (Abbott AxSYM System for HIV-1 and HIV-2, Abbott Laboratories). HIV-1 positive specimens were further characterized using the Calypte Aware BED EIA HIV-1 Incidence Test (Calypte Biomedical Corporation), which detects recent infection based on HIV-specific antibody affinity [8]. The cutoff for this assay was normalized optical density of  $\leq 0.8$  [9]. The  $\chi^2$  test was used for statistical analysis of BED Incidence Test data in the resistance study sample relative to the entire survey. Multivariate logistic regression analysis was done using data from all women aged  $\leq 30$  years in 8 surveys to explore the factors associated with recent infection. Variables available in the dataset included age, gravidity, year of survey, and province. Results were reported with odds ratios (ORs), 95% confidence intervals (CIs), and *P* values.

### Genotyping

Resistance genotyping was performed using remnant serum specimens stored at  $-70^\circ\text{C}$  following serological testing. Sequencing of the *pol* gene was done using an in-house assay certified by the Virology Quality Assessment program. In brief, a nested polymerase chain reaction (PCR) was performed using previously defined methods to generate a 1.7-kb amplicon spanning both the protease and reverse transcriptase genes [7]. In cases where there was no amplification of the *pol* gene, the protease and reverse transcriptase regions were amplified separately [10, 11]. Genotypic resistance was defined as the presence of resistance mutations using the Stanford Calibrated Population Resistance algorithm, version 4.1 beta [12, 13]. Specimen subtype was assigned using the same algorithm. Sequences were ordered according to date of collection and prevalence classification assigned according to the recommended

WHO method [5]. If no resistance was found within the first 34 specimens, prevalence was classified as low ( $<5\%$ ). If resistance was detected, then 47 sequences were evaluated. If the number of sequences with relevant resistance mutations was between 2 and 8, the prevalence of TDR was classified as moderate ( $5\%–15\%$ ).

## RESULTS

### Demographic Data From Amplified Specimens

Specimens were selected from 9 surveys conducted between 2005 and 2009 in GP and KZN (Table 1). A total of 1006 specimens were subjected to resistance testing, from which 354 analyzable sequences were obtained (35%). All specimens were subtype C, except for 1 subtype B (KZN-2008), 1 subtype A (GP-2009) and 1 subtype D (KZN-2009). In all surveys, the median age of women was between 19 and 21 years.

### Classification of Threshold Survey Sequence Data

Five surveys were conducted in GP utilizing 294 specimens, from which 196 sequences were obtained (67%). The PCR amplification rate ranged from 76% to 93% in 4 surveys but was lower in 2007 at 46%. In all surveys, the levels of TDR for the nucleoside reverse transcriptase inhibitor (NRTI) and nonnucleoside reverse transcriptase inhibitor (NNRTI) drug classes were  $<5\%$  [5]. In 2005, the protease gene was not analyzed; thus, a classification could not be made. In 2007, 1 specimen had the protease inhibitor (PI) mutation M46I. An additional sequence had 185V, which was not considered a surveillance drug resistance mutation (SDRM) at the time of this survey (GP-2007) and therefore was not included in the final analysis. Overall, levels of TDR for the PI class of drugs for 2006–2009 in GP were classified as low ( $<5\%$ ).

Four surveys were conducted in KZN. A total of 712 specimens were analyzed, from which 158 sequences were obtained (22%). PCR amplification rates were too low in 2005 (13%) and 2008 (14%) to allow us to reach the required number of sequences ( $n = 47$ ), and TDR classification was not possible in these years. In 2007, 67% of specimens were amplified; no resistance was detected in sequences from the first 34 specimens, allowing us to categorize transmitted resistance levels as low ( $<5\%$ ) according to the WHO method [5]. In the 2009 survey however, the presence of NNRTI mutations in 3 sequences resulted in a classification of moderate levels of TDR ( $5\%–15\%$ ) for the NNRTI class of drugs, whereas the levels were low ( $<5\%$ ) for the NRTI and PI drug classes. Interestingly, 2 sequences with NRTI mutations and 3 sequences with NNRTI mutations were identified in the preceding year. However, a threshold could not be calculated for the 2008 survey, because numbers of sequences obtained were insufficient to classify TDR based on the WHO method.

**Table 1. Transmitted HIV Drug Resistance Threshold Surveys Performed in Gauteng and KwaZulu-Natal Provinces, 2005–2009**

Province	Year	No. of Specimens Tested	No. of Sequences Analyzed	Amplification Rate	Median Age (Range), y	BED “Recent” Infections			HIV Subtype	No. With Mutations	Mutational Patterns			Threshold Level
						Entire ANSUR	Resistance Substudy	P Value			PI	NRTI	NNRTI	
Gauteng Province	2005	51	34	76%	21 (18–22)	100/1006 (11%)	7/34 (21%)	.080	C	0				<5% NRTI, NNRTI
	2006	40	34	93%	20 (18–21)	265/1923 (14%)	0/34 (0%)		C	0				<5% all drug classes
	2007	133	47	46%	20 (18–21)	572/2184 (26%)	17/47 (36%)	.125	C	1	M46I	M184I		<5% all drug classes
	2008	43	34	81%	20 (18–21)	264/2251 (12%)	9/34 (27%)	<b>.009</b>	C	0				<5% all drug classes
	2009	58	47	81%	19 (18–21)	245/2125 (12%)	10/47 (21%)	<b>.040</b>	C (1A)	1		M184V	Y188L	<5% all drug classes
KwaZulu-Natal Province	2005	287	40	14%	21 (18–24)	263/1359 (19%)	7/40 (18%)	.716	C	1			K101E Y181C	ND
	2007	61	34	67%	19 (18–22)	352/2715 (13%)	7/35 (20%)	.220	C	0				<5% all drug classes
											M46I			
												M184V	K103N	
	2008	284	37	13%	20 (18–24)	339/2676 (13%)	10/37 (27%)	.144	C (1B)	5		K219R		ND
													K103N	
													K103N	
													K103N	
	2009	80	47	71%	19 (18–21)	506/2692 (19%)	15/48 (31%)	<b>.029</b>	C (1D)	3			V106M	<5% PI, NRTI5-15% NNRTI
													K101P K103N	

Bold text indicates significant *P* values.

Abbreviations: ANSUR, annual antenatal survey, South Africa; BED, BED EIA HIV-1 Incidence Test; HIV, human immunodeficiency virus; ND, not determined due to insufficient No. of available specimens; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

## Mutational Patterns

A total of 11 of the 354 sequences (3%) were found to harbor resistance mutations (Table 1). The most common mutation in reverse transcriptase was K103N, which was present in 5 sequences, followed by M184V in 3 sequences. One sequence contained the SDRM K219R. For protease, 2 sequences had M46I [13].

## Use of Surrogate Markers to Assess Recent Infection

We further investigated whether specimens selected in the resistance survey were enriched for recent infections. In 7 of 9 surveys, the proportion of recently infected women was higher in the selected group relative to the entire survey population, although it was only significant in 3 of the later surveys because of small numbers (Table 1).

In order to determine whether the demographic criteria used for inclusion into TDR surveys were also supported by a classification of recent infection, a multivariate analysis was performed assessing data from all women  $\leq 30$  years of age participating in 8 of the 9 surveys. (GP-2005 was not included due to missing demographic data.) A total of 12 397 women were included in this analysis. Controlling for province, age, gravidity, and year was independently associated with being classified as recent infection by the BED Incidence Test. With each increasing year of age, women were 9% less likely to be classified as having a recent infection (OR, 0.91 [95% CI, .90–.92];  $P = .0000$ ). Similarly, women were 12% less likely to be classified as recent infection with each additional pregnancy (OR, 0.88 [95% CI, .82–.94];  $P = .0000$ ). There was also an association between recent infection and year of survey in that women were 6% less likely to be classified as recent infection for each year between 2005 and 2009 (OR, 0.94 [95% CI, .91–.97];  $P = .002$ ).

## DISCUSSION

We performed surveillance of TDR following the WHO-suggested method, using specimens from the ANSURs conducted by the South African National Department of Health. This study used specimens collected between 2005 and 2009 and focused on 2 provinces, both with high HIV prevalence estimates [3]. Our results indicated that the levels of TDR were  $< 5\%$  for all drug classes during this period in GP. In KZN, levels were low in 2007 for all drug classes; they appeared to be increasing in 2009 for NNRTIs, as the KZN-2009 survey was classified as having 5%–15% transmitted NNRTI resistance.

This report is an update of an earlier publication reporting low levels of TDR in ANSURs performed in GP in 2002 and 2004 [7]. Since this previous report, the national treatment program has initiated ART in nearly 1 million HIV-infected

individuals [3]. Single-dose nevirapine, to prevent mother-to-child transmission, was also in use during the time of these surveys. Our results suggest that the levels of TDR in GP remained unchanged at  $< 5\%$  until 2009, as all surveys from this province were classified as low for all 3 drug classes. However in KZN, there was an indication that TDR may be increasing for the NNRTI drug class, although only 2 of the 4 surveys were evaluable. The 2007 survey showed levels of  $< 5\%$ , while the 2009 survey showed levels of 5%–15% for the NNRTI drug class. While insufficient sequences precluded analysis for TDR in 2008, the presence of 5 sequences with mutations supports the notion that resistance may have been increasing before 2009. It will be important to verify this finding by performing follow-up surveys in 2010 in KZN, perhaps including additional sites.

One of the limitations of the study was the low frequency of PCR amplification of specimens, particularly those from KZN, which compromised the assessment of the 2005 and 2008 surveys. This is probably because these remnant serum specimens were not adequately stored for optimal preservation of viral RNA needed for resistance testing. Although unlikely to compromise the interpretation of the data, the low amplification rate meant that high volumes of specimens had to be tested. Despite this, there were insufficient sequences available to perform TDR surveys in both 2005 and 2008. Since the amplification rate was considerably lower in KZN than GP, this suggests that logistical issues in specimen collection and handling in KZN (confounded by the need to transport the specimens to the drug resistance testing lab in GP) should be examined particularly in light of the possibility that additional surveys may need to be conducted in this province.

K103N and M184V were the most common mutations detected. These mutations are associated with TDR and are commonly found in patients failing first-line therapies in South Africa [14–17]. K103N and M184V occur rarely in untreated individuals [13] and are selected for by nevirapine/efavirenz and lamivudine, respectively, causing high-level resistance to these drugs. Thus, these women were either exposed to anti-retroviral drugs or infected with a resistant strain. It is also possible that the K103N mutation arose due to single-dose nevirapine exposure despite no record of prior pregnancy. K219R is listed as an SDRM for the purposes of transmitted resistance, although the effect on NRTI susceptibility is unknown. The M46I mutation found in 2 individuals is a PI resistance-associated mutation that occurs at very low frequency ( $< 0.2\%$ ) among drug-naïve persons. M46I can be polymorphic; its presence may not signify TDR, especially because PI-based regimens are reserved for second-line treatment, and other PI-associated mutations were not observed in this specimen.

While the ANSUR has been useful as a minimum resource method for examination of TDR in resource-limited settings,

the issue of whether demographic criteria are reliable surrogate markers of recent infections remains open to discussion. We found that specimens selected for these surveys were enriched for individuals with recent infections as defined by BED EIA HIV-1 Incidence Testing, although results were statistically significant in only 3 surveys (probably due to the small sample sizes). However, a multivariate analysis involving >12 000 women showed that young age and first gravidity were significantly associated with a classification of recent infection. Despite the recommendation that the predictive value of this assay is too low to classify recent infection on an individual basis [18], our initial assessment is that the BED Incidence Test may be an additional useful measure to include when developing inclusion and exclusion criteria for population-based surveys of TDR.

TDR in resource-limited countries, such as South Africa, is not unexpected. Increased levels have been detected over the years in Europe and the United States [19, 20] and more recently in Uganda, where ART programs have been in operation for a longer time [21]. Because South Africa's national ART program began in 2004, it is not unexpected that earlier surveys showed low rates of TDR. However, this report suggests that increasing levels of transmitted NNRTI-resistant virus may be occurring in KZN. This report must be treated with caution, and ongoing vigilance is required. TDR surveillance should be repeated in KZN and results confirmed. A systematic and standardized assessment of factors occurring within the ART delivery program, which may favor the selection of drug-resistant virus in populations receiving care and its subsequent transmission to newly infected individuals, needs to be implemented.

## Notes

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